J. Clin. Chem. Clin. Biochem.
Vol. 27, 1989, pp. 201–204

Measurements of Enzyme Activity and Immunoreactivity of Plasma Renin[1]) Limitations of the Conventional Method for Assay of Plasma Renin Catalytic Activity

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(Received September 6, 1988/January 9, 1989)

Summary: The values for "plasma renin activity", "plasma renin concentration" (both determined by the rate of angiotensin I generation)[2]) and the plasma active renin protein mass concentration (active enzyme protein measured by a direct radioimmunoassay) were compared. The values for "plasma renin activity" (angiotensin I generation from endogeneous renin substrate) and "plasma renin concentration" (angiotensin I generation from exogeneous excess of renin substrate) showed a significant correlation. However, the correlation was lost in samples with high (plasma renin activity $\geqq 10$ μg/l · h) and low (plasma renin activity $\leqq 1$ μg/l · h) renin contents. In another study, values for "plasma renin activity" were correlated with those for plasma active renin protein mass concentration, and values for "plasma renin concentration" were also correlated to those for plasma active renin protein mass concentration. However, in samples with low renin contents (plasma active renin protein mass concentration $\leqq 20$ ng/l), there was no correlation between values for "plasma renin activity" and plasma active renin protein mass concentration, although those for "plasma renin concentration" and plasma active renin protein mass concentration showed a significant correlation. These results suggest that the current method for the assay of plasma renin activity gives erroneous values for low and high plasma renin concentrations. The new radiometric method for the determination of the plasma active renin protein mass concentration seems to be applicable to plasma samples with a wide range of renin concentrations.

Introduction

Measurement of plasma renin is very important in clinical management of hypertension and in basic research. Plasma renin is usually assayed as plasma renin activity, and the resulting values are important in clinical diagnosis (1–4). However, the values of plasma renin activity for plasma samples with high renin contents may be erroneous, because the intrinsic amount of substrate of renin may be insufficient (5, 6). An improved assay of "plasma renin concentra-

[1]) Enzyme: Renin, EC 3.4.23.15

[2]) Managing Editor's remarks
Plasma renin activity ("PRA") and plasma renin concentration ("PRC") are both determined by radioimmunoassay of angiotensin I liberated from angiotensinogen by renin.
The former method ("PRA") uses the intrinsic (endogeneous) renin substrate; the latter uses extrinsic (exogeneous) excess of renin substrate, resulting in higher values ("PRC"). Both methods are based on the assay of angiotensin I as the enzymatic product of renin action; therefore they are to be considered both as methods estimating renin *catalytic* activity *concentration*. None of them measures plasma renin concentration ("PRC") in contrast to the immunological measurement of plasma active renin protein mass concentration. It is therefore strongly recommended that the term "plasma renin concentration (PRC)" be avoided in future, in order to differentiate clearly between *renin catalytic activity concentration* and *active renin protein mass concentration*, thus also following the recommendation of the IUB and IFCC.

tion", based on the same principles, has been developed, in which extrinsic substrate is added; the effect of adding substrate to samples from different species has not yet been examined (5, 6). Furthermore, no standard method for "plasma renin concentration" assay has yet been established. Very recently, *Ménard* et al. (7) reported the direct radiometric assay of plasma active renin protein mass using a monoclonal antibody. In the present study, we compared the values for renin obtained by these three assays. The results indicated certain limitations of "plasma renin activity" determination, and demonstrated the value of the new radiometric enzyme protein assay for clinical purposes.

Subjects and Methods

Study I

Plasma samples were collected from 73 normal and hypertensive outpatients of the Department of Geriatric Medicine, Osaka University Hospital, Osaka. The subjects were made to sit for 30 min and then 5 ml of blood was taken from the antecubital vein into a cold glass tube containing EDTA · 2Na (2.7 mmol/l blood). The blood was promptly centrifuged at 2500 min^{-1} for 30 min, and the plasma was stored at −60 °C till the assay. Both enzymatic assays for plasma renin were performed simultaneously within 2 weeks after plasma sampling.

Study II

Totals of 41 and 42 plasma samples were collected for comparison of the values for plasma renin catalytic concentration (exogeneous substrate), plasma active renin protein mass concentration, plasma renin catalytic concentration (endogeneous substrate) and plasma active renin protein mass concentration. These samples were all obtained from hypertensive subjects who had remained in the supine position for 30 min. The samples were centrifuged and the plasma was stored as for study I. The enzymatic assays for plasma renin and the immunological assay of plasma active renin protein mass concentration were performed within 2 weeks after plasma sampling.

The sodium intake of all subjects was approximately 100 mmol/d. Informed consent to participate in the study was obtained from each subject after full explanation of the study. The protocol was approved by the Human Research Committee of the Department of Geriatric Medicine, Osaka University.

Plasma renin catalytic activity concentrations were measured as rates of angiotensin I generation by the methods of *Ikeda* et al. ((8), intrinsic substrate) and *Goto* et al. ((5), extrinsic substrate), respectively. The plasma active renin protein mass concentration was measured with a commercially available renin immunometric assay kit (Diagnostics Pasteur, 92430-Marnes La Coquette, France) according to *Ménard* et al. (7). This assay, which uses a monoclonal antibody, is highly specific for human active renin (7, 9). In our laboratory, the minimal concentration of renin detected by this method was 3 ng/l, and the intra- and inter-assay coefficients of variation (CV) were 3.1−7.9% (n = 10) and 4.0−9.8% (n = 10), respectively. The recovery of a known amount of renin was 91−112% (n = 8).

Results

The enzymatic activity concentrations of plasma renin of the 73 samples measured with both angiotensin I generating methods showed a significant correlation ($y = 4.66x - 0.93$, where y is plasma renin determined with extrinsic excess of renin substrate and x is plasma renin assayed with intrinsic substrate; $n = 73$; $r = 0.81$; $P < 0.01$), as seen in figure 1. However, the values for plasma renin measured with exogeneous substrate were obviously higher than expected from the regression equation in samples with over 10 µg/l · h of plasma renin assayed with endogeneous substrate. Moreover, in samples with levels of plasma renin < 1 µg/l · h, there was no correlation between values obtained by both enzymatic methods.

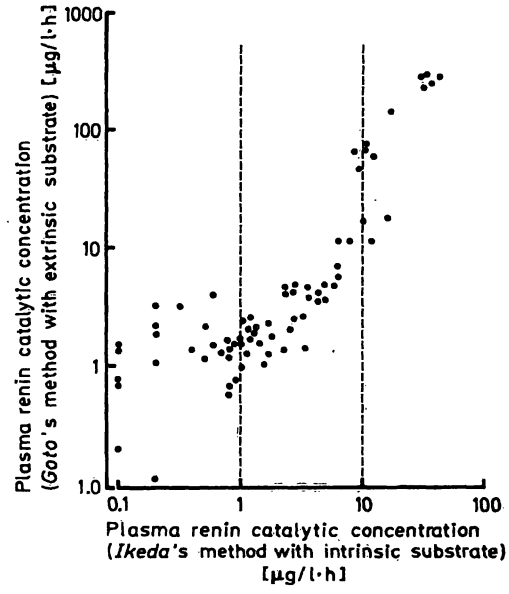

Fig. 1. Correlation between values of plasma renin catalytic activity concentration, measured by the method of *Ikeda* et al. (8) and the method of *Goto* et al. (5). n = 73

The correlations between plasma active renin protein mass concentration and plasma renin catalytic activity concentrations are shown in figure 2. Values for plasma renin (measured with endogeneous substrate, y) were correlated with those of plasma active renin protein mass concentration (x): $y = 0.08x - 0.75$; $r = 0.97$; $n = 41$; $P < 0.01$. Values of plasma renin (measured with exogeneous substrate, y) were also correlated with those of plasma active renin protein mass concentration (x); $y = 0.31x - 1.9$; $r = 0.98$; $n = 42$; $P < 0.01$. However, there was no correlation between values for plasma active renin protein mass concentration and plasma renin (measured with en-

dogeneous substrate, n = 15) in samples with plasma active renin protein mass concentration values of under 20 ng/l, whereas plasma renin (measured with exogeneous substrate, y) showed a significant correlation with plasma active renin protein mass concentration (x) in samples with low renin contents: $y = 0.21x + 0.09$; $r = 0.81$; $n = 18$; $P < 0.01$.

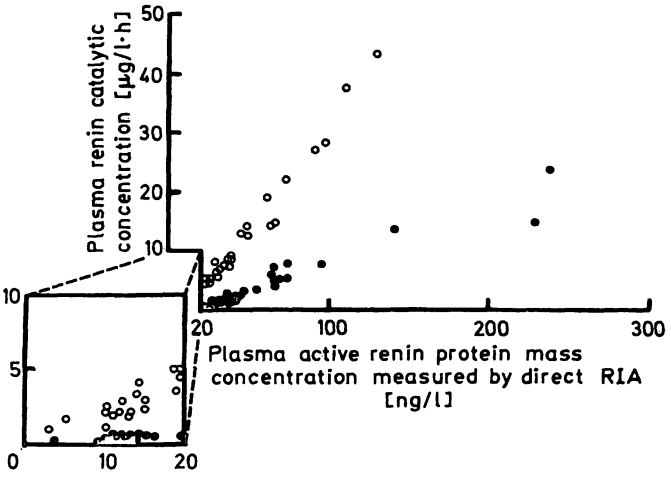

Fig. 2. Correlations of plasma active renin protein mass concentrations measured by immunometric assay and enzymatic activity concentrations of plasma renin.
o plasma "renin concentration", n = 42; method of *Goto* et al. (5) with extrinsic substrate
• plasma "renin activity", n = 41; method of *Ikeda* et al. (8) with intrinsic substrate

Discussion

"Plasma renin activity" is commonly used for measurement of renin in the management of patients with hypertension and diseases causing fluid-electrolyte impairment, such as *Bartter*'s syndrome. Because the "plasma renin activity" changes with the plasma content of substrate for renin, values for "plasma renin activity" and "plasma renin concentration" (measured with excess of exogeneous renin substrate) show discrepancies in some conditions such as pregnancy, liver cirrhosis and high renin hypertension (5, 6, 10). In the present study, the correlation between both enzymatic methods was abnormal in samples with a high renin content (plasma renin measured with endogeneous substrate $\geqq 10$ μg/l · h) and no correlation was observed in those with a low renin content (plasma renin measured with endogeneous substrate $\leqq 1$ μg/l · h). In those with high renin contents, the abnormality may have been due to an insufficient concentration of substrate. However, the reason for the absence of a correlation in samples with low renin contents is unknown. The most probable explanation is that the assay performance of "plasma renin activity" methods are poor near the detection limit. The effect of cryoactivation of prorenin to renin during the storage of plasma on the values obtained by all three methods is negligible, because samples stored frozen under −40 °C exhibit no activation of prorenin, as formerly reported by *Sealey* et al. (11). Figure 2 shows that the correlations between plasma active renin protein mass concentration and plasma renin measured with both enzymatic methods are good, except that the correlation between plasma active renin protein mass concentration and plasma renin measured with endogeneous substrate is lost at plasma active renin protein mass concentration values less than 20 ng/l. These data suggest that values for plasma renin measured with endogeneous substrate are not accurate in samples with low renin contents (plasma renin assayed with endogeneous substrate < 1 μg/l · h) or high renin contents (plasma renin assayed with endogeneous substrate > 10 μg/l · h). These data are consistent with a report by *Dessi-Fulgheri* et al. (12) that plasma renin measured with endogeneous substrate and plasma active renin protein mass concentration measured by direct RIA are not correlated in low renin plasmas (plasma renin assayed with endogeneous substrate < 2 μg/l · h or plasma active renin protein mass concentration < 40 μg/l); and with the report of *Morganti* et al. (9) that plasma angiotensin II concentration is better correlated with plasma active renin protein mass concentration than with plasma renin determined with endogeneous substrate. These results are clinically significant, because "plasma renin activity" (measured with endogeneous substrate) is often used as a diagnostic criterion in low renin conditions such as primary aldosteronism (1, 2, 4). The low sensitivity and specificity of this test might be due to its poor quantitativity at low renin concentrations. Under these condition, the detection limit must be increased substantially by prolonging the incubation time so that the amount of angiotensin I developed can be easily measured by RIA. However, the assay is not modified in this way in clinical laboratories.

The present study showed that values for plasma renin determined with exogeneous substrate and plasma active renin protein mass concentration determined by direct RIA were well correlated. Furthermore, one of the advantages of the direct RIA method is that values for plasma active renin protein mass concentration obtained in different laboratories can be standardized. Thus this new radiometric assay of plasma active renin protein mass concentration seems useful as a laboratory test of plasma renin.

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References

1. Hiramatsu, K., Yamada, T., Yukimura, Y., Komiya, I., Ichikawa, K., Ishihara, M., Nagata, H. & Izumiyama, T. (1981) A screening test to identify aldosterone-producing adenoma by measuring plasma renin activity: Results in hypertensive patients. Arch. Intern. Med. *141*, 1589–1593.
2. Lyons, D. F., Kem, D. C., Brown, R. D., Hanson, C. S. & Carollo, M. L. (1983) Single dose captopril as a diagnostic test for primary aldosteronism. J. Clin. Endocrinol. Metab. *57*, 892–896.
3. Muller, F. B., Sealey, J. E., Case, D. B., Atlas, S. A., Pickering, T. G., Pecker, M. S., Preibisz, J. J. & Laragh, J. H. (1986) The captopril test for identifying renovascular disease in hypertensive patients. Am. J. Med. *80*, 633–644.
4. Lins, P. E. & Adamson, U. (1986) Plasma aldosterone-plasma renin activity ratio: A simple test to identify patients with primary aldosteronism. Acta Endocrinol. *113*, 564–569.
5. Goto, T., Imai, N., Hirose, S. & Murakami, K. (1984) An improved method for determination of active and total renin concentration in human plasma using an excess of sheep substrate. Clin. Chim. Acta *138*, 87–98.
6. Derkx, F. H. M., Stuenkel, C., Schalekamp, M. P. A., Visser, W., Huisveld, I. H. & Schalekamp, M. A. D. H. (1986) Immunoreactive renin, prorenin, and enzymatically active renin in plasma during pregnancy and in women taking oral contraceptives. J. Clin. Endocrinol. Metab. *63*, 1008–1015.
7. Ménard, J., Guyenne, T.-T., Corvol, P., Pau, B., Simon, D. & Roncucci, R. (1985) Direct immunometric assay of active renin in human plasma. J. Hypertens. *3* (suppl. 3) S275–S278.
8. Ikeda, I., Iinuma, K., Takai, M., Yanagawa, Y., Kurata, K., Ogihara, T. & Kumahara, Y. (1981) Measurement of plasma renin activity by a simple solid phase radioimmunoassay. J. Clin. Endocrinol. Metab. *54*, 423–428.
9. Morganti, A., Turolo, L., Pulazzini, E. & Zanchetti, A. (1987) Comparative measurements of immunoreactive renin, plasma renin activity and angiotensin II in human plasma. Clin. Exper. Hypertens. *A9*, 1367–1381.
10. Degos, F., Benhamou, J. P., Menard, J., Salmon, J. & Raynaud, J. P. (1978) Plasma renin substrate sensitivity to oestrogen and oestrogen metabolism in cirrhosis. Eur. J. Clin. Invest. *8*, 243–248.
11. Sealey, J. E., Moon, C., Laragh, J. H. & Alderman, M. (1976) Plasma prorenin: Cryoactivation and relationship to renin substrate in normal subjects. Am. J. Med. *61*, 731–738.
12. Dessì-Fulgheri, P., Cocco, F., Gloriosso, N., Bandiera, F., Madeddu, P. & Rappelli, A. (1987) Immunoradiometric assay of active renin in human plasma: comparison with plasma renin activity. Clin. Exper. Hypertens. *A9*, 1383–1390.

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